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ORIGINAL PAPER

Seasonal variation of microelement contents in leaves of *Cyclocarea paliurus* among the provenances

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Abstract: *Cyclocarya paliurus* as a multiple function plant can accumulate biologically important microelement elements. To reveal the variation of selected microelement concentrations in leaves of *C. paliurus* provenances during the growing season, 12 *C. paliurus* provenances in the field trial were sampled five times at approximately 1-month intervals. The method of inductively coupled plasma optical emission spectrometer (ICP-OES) was employed to determinate average concentrations of Fe, Mn, Zn, Cu and Se in leaves of 12 *C. paliurus* provenances. The results show that on average, the concentrations of five microelement in the leaves follows an order of Fe > Mn > Zn > Cu > Se. Variance analysis shows that there are significant differences in Fe, Mn and Zn concentrations among the twelve provenances ($p<0.05$), while there is no significant difference between Cu and Se concentrations. A significant difference was also observed in the concentrations of five microelements at the different sampling times ($p<0.001$), but the mean concentrations for each microelement showed different temporal dynamic patterns. Meanwhile, a significant correlation between concentrations of Se and other measured microelements was detected in the leaves of *C. paliurus*, except for Mn. Obtained results not only demonstrated that leaves of *C. paliurus* exhibited higher levels of microelements (Fe, Mn, Cu, Zn and Se), but also provided a basis for breeding strategies of superior provenances with rich content of microelements, and choosing optimum harvesting time for food industry in future.

Keywords: *Cyclocarea paliurus*; mineral nutrition; provenance; concentration; ICP-OES (coupled plasma optical emission spectrometer)

Introduction

Mineral elements play important physiological roles in both the human body and plants. It has been estimated that iron (Fe) and zinc (Zn) deficiencies are damaging the health of one-third of the world's population (Hotz and Brown 2004). Globally, 0.5 and 1 billion people have inadequate intakes of selenium (Se), which include populations in the developed countries such as Western Europe (Combs 2001). In the past decade, lots of interests were paid to developing varieties of grain crops with enhanced concentrations of elements (such as Fe, Se and Zn) to improve the nutritional quality of grain (White and Broadley 2005; Cakmak 2008; Lu et al. 2008; Wissuwa et al. 2008; Jiang et al. 2008), while only some mineral compositions or contents were reported in medical woody plants (Lovkova et al. 2001; Hardisson et al. 2001; Osaki et al. 2003; Xie et al. 2006; Thomidis et al. 2007;). Medicinal plants can accumulate biologically important elements, such as manganese (Mn), Fe, copper (Cu), Se, and Zn, and the contents of these elements in various plants are different (Lovkova et al. 2001). The accumulation of individual elements in medicinal plants is correlated to synthesis of physiologically active substances (PAS) and their pharmacological properties with chemical peculiarities, whereas therapeutic effects of medicinal plants were associated with their chemical peculiarities (Lovkova et al. 2001). In respect to their therapeutic effects, individual elements in medicinal plants are of particular interest, such as, alkaloids, terpenoids (triterpene and steroid saponins), phenolic compounds, glycosides (cardiac glycosides), and polysaccharides (Jiang et al. 2006; Ding et al. 2007; Sultana and Anwar 2008; Xie et al. 2010; Fang et al. 2011). However, the ability of different medicinal plants to concentrate elements, as well as their role as regulators of PAS metabolism is different. Thus, understanding of the chemical features of medicinal plants and

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their pharmacological properties is very important.

Cyclocarya paliurus (Batal) Iljinskaja, as a medicinal plant and fine timber, is native to China, and the sole species in its genus (Fang et al. 2006). The leaves of *C. paliurus* have been a food sources for maritime people for a long time (Birari and Bhutani 2007), and also been used as drug formulations in traditional Chinese medicine (TCM) as well as an ingredient in functional foods in China (Xie et al. 2010). Recent studies indicated that the leaves of *C. paliurus* have beneficial effects on the prevention of hypolipidemic and diabetes mellitus (Kurihara et al. 2003a; Kurihara et al. 2003b). Extracts from leaves of *C. paliurus* had strong inhibition against PTP1B (protein tyrosine phosphatase non-receptor type-1B), (Zhang et al. 2010) and inhibited PL (Phospholipase) activity (Birari and Bhutani 2007). Moreover, many other functions of *C. paliurus*, such as the improvement of mental efficiency, antihypertensive action and immunomodulation, have also been reported (Jiang et al. 2006; Xie et al. 2006). All these studies provide the basis for rapidly increasing interest in the use of *C. paliurus* as functional food ingredients and/or as traditional Chinese medicine. Most studies on *C. paliurus* were focused on the extract activities and low molecular weight substances, such as triterpenoids, flavonoids, steroids, saponins and other compounds in this plant (Xie et al. 2010), while a less attention was paid to developing the plantation resources of *C. paliurus* (Fang and Fu 2007). Duo to the multiple beneficial effects to human health, a huge production of leaves of *C. paliurus* is required for tea production and medical uses. However, there are not enough *C. paliurus* plantations for leaf production (Fang et al. 2006; Fang et al. 2007). Consequently, recently lots of attentions are paid to developing plantations of *C. paliurus* as an ingredient to be used in traditional Chinese medicine or functional food.

The content of mineral elements in plants is affected by genetic, cultural, harvesting and climatic factors that occur during the plant growing period (Oury et al. 2006; Thomidis et al. 2007; Jiang et al. 2008; Zhao et al. 2009). Knowledge of seasonal accumulation of mineral nutrients and their genetic variation is necessary to develop criteria for optimum harvest times and selecting superior varieties for both yield and quality of medical plants. However, no information is available on the influence of harvesting time, cultural and genetic factors on mineral element contents of *C. paliurus* leaves. The objectives of this study were to investigate the variation in concentration of some important microelements (Fe, Mn, Cu, Zn and Se) in the leaves of *C. paliurus* from different provenances at various harvesting (sampling) times. The results of the study would provide new information for both determination of harvesting time and future breeding for microelement-rich cultivars of *C. paliurus*.

Materials and methods

Plant material and experimental design

Seeds from 12 provenances of *C. paliurus* in China (Fig. 1) were collected from October to November in 2006. The detail geographical and climatic information of the 12 provenances of *C. paliurus* in the experiment was shown in Table 1. After drying in open sunlight and removal of unwanted materials, the seeds from each provenance were put into an open plastic box in the laboratory until required. To overcome the dormancy and achieve rapid, uniform and high germination rates, seeds of each *C. paliurus* provenance were subjected to chemical scarification, exogenous GA_3 treatments and stratification treatments in early January 2007 according to the method proposed by Fang et al. (2006). After 3-month stratification treatment, the germinated seeds were sown in the containers until no germinated seeds were observed. The seedlings in the containers were transplanted into the field in Zhenjiang nursery in early June 2007, and planting space was 40 cm × 40 cm.

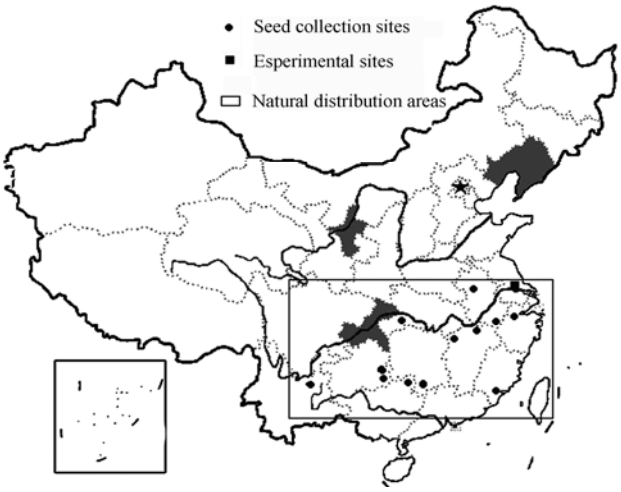

Fig. 1 The seed collection sites of *Cyclocarea paliurus* in this study

The provenance experiment with 1-year-old seedlings in 12 plots was established in middle March 2008. The planting space was 100 cm×120 cm and the provenance units were represented by 48–168 plants, which were distributed randomly in the field. The experimental area (27°40′ N latitude, 108°26′ E longitude) is also located at Zhenjiang nursery (Fig. 1). The average annual temperature is 14.1°C and the frost-free period is 220 days. The average annual precipitation ranges from 1000 to 1100 mm, but most of the precipitation is distributed from June to September, accounting for 60% of the total. The soil at the depth of 0 to 40 cm is a sandy loam with an organic matter content of 1.77%, available nitrogen content of 77 mg·kg^{-1}, and available P and K contents of 14.9 and 132.6 mg·kg^{-1}, respectively.

Sample collections

Fully developed leaves of approximately 50-g fresh weight of each provenance were sampled at about 1-month intervals (25 June, 27 July, 20 August, 25 September, and 25 October in 2008), and totally 60 samples were collected. The collected fresh samples were washed with distilled water in order to remove the dust. All samples were dried, sliced and ground into fine powder before extraction. Then, samples were stored at room temperature until analysis.

Extraction and determination of selected microelements

Combination with sulfuric and perchloric acids was used for sample digestion. Each dry sample (1.0 g) was precisely weighed and put into a beaker, while 98% sulfuric acid of 9 mL and 70% perchloric acid of 1 mL were added. The mixture was allowed to stand overnight, and then the beaker was heated electrically to decompose the samples until the solution became clear. After the clear solution was continuously heated for another 20 min and cool down, the solution was adjusted to a volume of 100 mL for determination of microelements. The concentrations of microelements including Fe, Zn, Mn, Se and Cu were determined using Inductively Coupled Plasma Optical Emission Spectrometer (Optima 4300DV ICP-OES, PerkinElmer Inc. Wellesley, MA, United States). Three replications were made in the analysis of each sample.

Table 1. Geographical and climatic information of the 12 provenances of *Cyclocarea paliurus* used in the experiment

Provenance name	Province	County or location	Latitude (N)	Longitude (E)	Altitude (m)	Age of stand (year)	Precipitation (mm)	Annual mean temperature (°C)
FZP	Fujian	Zhangpu	24°07′	117°37′	750	uneven	1500.0	21.0
HJH	Hunan	Jianghua	24°54′	111°42′	650	uneven	1540.0	17.8
YKM	Yunnan	Kuenming	25°02′	102°44′	1913	uneven	1000.6	14.5
GZY	Guangxi	Zhiyuan	26°02′	110°40′	800	30	1761.1	16.4
GLP	Guizhou	Liping	26°13′	109°18′	750	uneven	1325.9	15.6
GJH	Guizhou	Jianhe	26°31′	108°42′	920	uneven	1400.0	14.5
JXS	Jiangxi	Xiushui	29°01′	114°30′	500	uneven	1450.0	16.5
JLS	Jiangxi	Lushan	29°33′	116°30′	1000	uneven	1917.0	11.7
HHF	Hubei	Hefeng	29°48′	110°11′	747	uneven	1400.0	15.8
AQLF	Anhui	Qingliangfeng	30°07′	118°51′	1100	uneven	1480.0	11.3
ZAJ	Zhejiang	Anji	30°41′	119°41′	400	uneven	1476.0	14.5
ASC	Anhui	Shucheng	31°02′	116°32′	704	uneven	1027.7	15.5

Statistical analysis

Statistical analysis was performed with Software SPSS 13.0 (SPSS Inc., Chicago, Illinois, USA). A GLM univariate analysis of variance (ANOVA) was conducted to compare individual microelement level by provenances and sampling times (based on Type III sums of squares), while Duncan's test was performed to detect differences between individual treatment level means. All statistical analyses were performed at a 95% confidence level. Associations among different microelements in the leaves of *C. paliurus* were determined by using Pearson's correlation analysis.

Results

Variation of microelement concentrations in different provenances

The mean concentrations of iron (Fe), manganese (Mn), zinc (Zn), copper (Cu) and selenium (Se) in leaves from 12 *C. paliurus* provenances are shown in Fig. 2. On average, the five microelement concentrations in the leaves follows an order of Fe > Mn > Zn > Cu > Se. The mean concentrations of Fe, Mn, Zn, Cu and Se for all provenances at five sampling times are 0.4885, 0.0505, 0.0299, 0.0133 and 0.0076 $mg \cdot g^{-1}$, respectively, while the ranges of the concentrations are 0.3454–0.8952, 0.0376–0.0593, 0.0263–0.0383, 0.0120–0.0141 and 0.0056–0.0106 $mg \cdot g^{-1}$, respectively. Variance analysis shows that there are significant differences among Fe, Mn and Zn concentrations form the twelve provenances ($p < 0.05$), while no significant difference in Cu and Se concentration is detected (Table 2). Compared to the average concentration of Zn from 12 provenances (about 0.0299 $mg \cdot g^{-1}$), Zn concentration of four provenances (ASC, GJH, AGLF and GLP) is increased by 28.2%, 7.7%, 3.1% and 2.7%, respectively, while Zn concentration of eight provenances (YKM, HJH, GZY, JXS, FZP, HHF, ZAJ and JLS) is decreased by 12.1%, 8.7%, 7.4%, 6.6%, 3.2%, 2.6%, 0.32% and 0.2%, respectively. Furthermore, Duncan's test indicated that Fe and Zn concentrations had no significant differences between provenances in most cases, except for Fe in HJH and JXS provenances and Zn in ASC provenance (Fig. 2), while concentration of Mn has a significant difference between the provenances ($p < 0.05$).

Table 2. Results of the quantities of microelements produced by the leaves of *Cyclocarea paliuru* from twelve provenances and at five sampling times by GLM univariate ANOVA in 2008

Source	Statistical index	Microelements				
		Fe	Mn	Cu	Zn	Se
Model	d*f*	16	16	16	16	16
	F	72.80	181.66	706.73	177.47	47.95
	P	<0.001	<0.001	<0.001	<0.001	<0.001
Provenance	d*f*	11	11	11	11	11
	F	7.09	4.84	1.72	2.52	1.33
	P	<0.001	<0.001	0.100	0.015	0.243
Sampling time	d*f*	4	4	4	4	4
	F	36.26	11.78	163.39	13.83	67.53
	P	<0.001	<0.001	<0.001	<0.001	<0.001

Notes: Degrees of freedom (df), *F*-statistic (*F*) and probability level (*P*) are shown. Statistically significant probabilities are shown in bold.

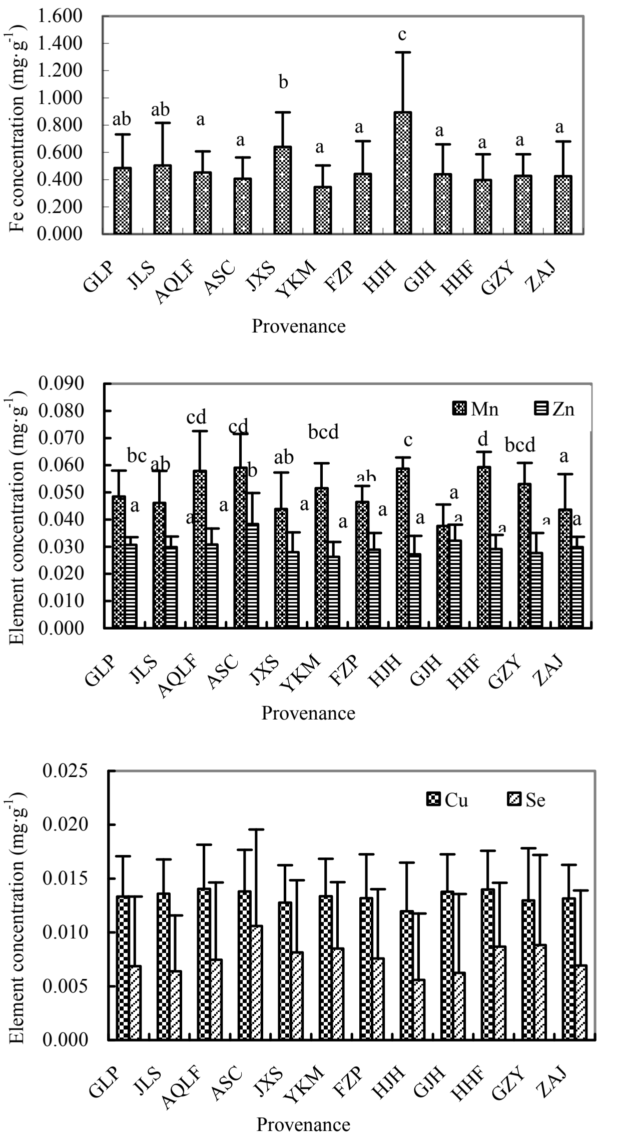

Fig. 2 Microelement concentrations in leaves of various *Cyclocarea paliurus* provenances (mean ± standard deviation). Different small letters indicate significant differences between various provenances for each microelement (p <0.05 by Duncan's test). Each value represents the mean of five sampling times.

Seasonal variation of microelement concentrations

The seasonal fluctuations in microelement concentrations in leaves of *C. paliurus* are presented from twelve provenances in Fig. 3. The mean concentrations of microelements in the leaves of *C. paliurus* showed different temporal dynamic patterns (Fig. 3) for the twelve provenances. Variance analysis showed that there are significant differences (p < 0.001) in the five microelement concentrations at five sampling times (Table 2). The highest concentrations of Fe and Zn were in July, and the lowest was in October for Fe and in June for Zn, respectively. However, the highest and lowest concentrations of Cu and Se were in September and in June (Fig. 3). The seasonal variation of Mn concentrations had a slight difference with the lowest concentrations in August and the highest in October. Furthermore, Duncan's test indicated that there were significant differences in microelement concentrations at different sampling times (Fig. 3).

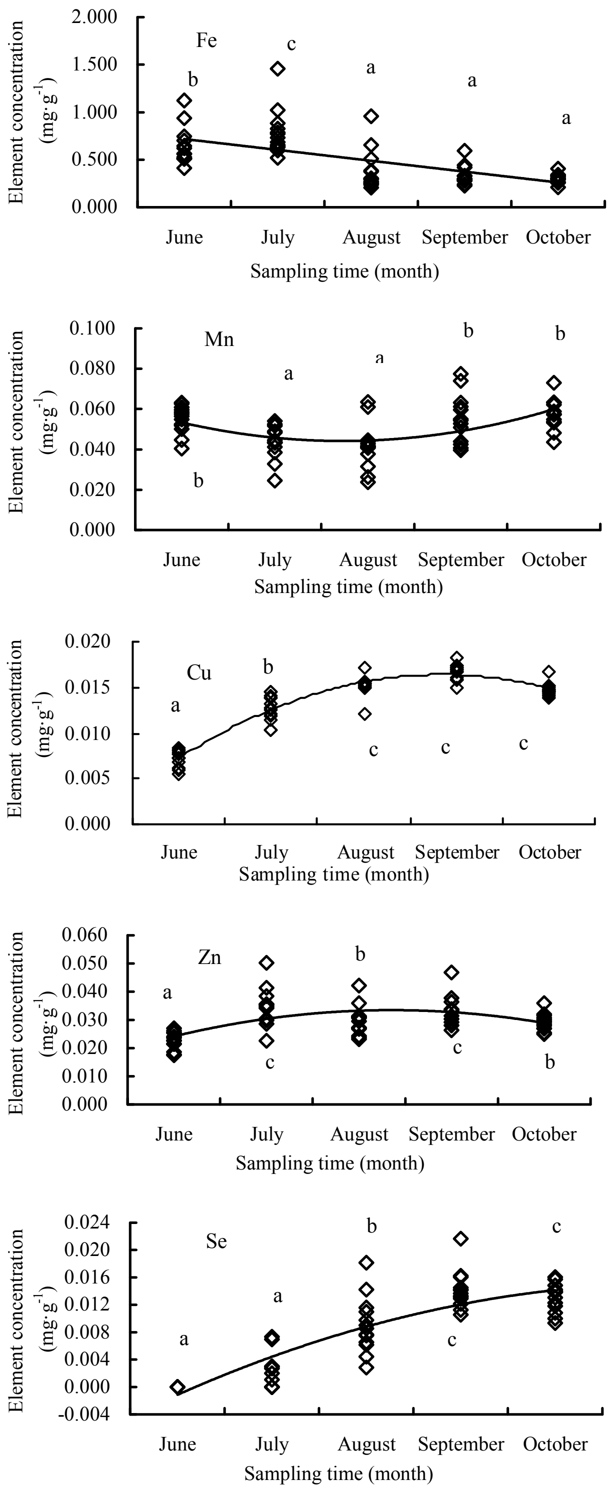

Fig. 3 Variation of microelement concentrations in leaves of 12 *Cyclocarea paliurus* provenances at different sampling times (mean ±standard deviation). Different small letters indicate significant differences between the sampling times for each microelement (p < 0.05 by Duncan's test).

The ranking of Cu concentration from 12 provenances at different sampling times was 0.0167[c] mg·g^{-1} in September >0.0153[c] mg·g^{-1} in August > 0.0148[c] mg·g^{-1} in October > 0.0127[b] mg·g^{-1} in July > 0.0072[a] mg·g^{-1} in June (where different superscript letters indicate significant difference at $p < 0.05$), while Mn concentration was 0.0574[c] mg·g^{-1} in October > 0.0550[b] mg·g^{-1} in September > 0.0549[a] mg·g^{-1} in June > 0.0436[a] mg·g^{-1} in July > 0.0416[a] mg·g^{-1} in August. Ranking of the Se concentration at different sampling times was 0.0141[c] mg·g^{-1} in September > 0.0131[c] mg·g^{-1} in October > 0.0090[b] mg·g^{-1} in August > 0.0020[a] mg·g^{-1} in July > 0.0000[a] mg·g^{-1} in June. The concentration of Fe in July was 50% higher than that in following months (August, September and October), while the concentration of Zn in July was 15.0%, 4.1% and 16.0% greater than that in August, September and October, respectively. Our results indicated that the concentrations of selected microelements in the leaves of *C. paliurus* were significantly influenced by the growing season, but in most cases this effect was not significant between September and October (p<0.05), except for Zn (Fig. 3).

Correlation between concentrations of different microelements

In order to determine the possible association between the concentrations of different microelements in the leaves of *C. paliurus*, the data collected (n=60) were processed with Pearson's correlation coefficients. The results indicated that Fe concentration was negatively correlated with Cu and Se concentrations (p<0.01), while the Cu concentration was positively correlated with concentrations of Zn and Se respectively (Table 3). Meanwhile, a positively significant correlation between Se and Zn concentrations was also observed in the *C. paliurus* leaves (Table 3). Furthermore, there is a good correlation between concentrations of Se and other measured microelements in the leaves of *C. paliurus* at the present study, and the Pearson's correlation coefficients of Se concentration with Fe, Mn, Cu and Zn were -0.642, 0.241, 0.805 and 0.368 respectively (Table 3), suggesting that the Se concentration could be a good indicator for selection of microelement-rich cultivars in future breeding system of *C. paliurus*.

Discussion

Cyclocarya Paliurus, one of woody medicinal plants, has been widely utilized in China both as drug formulations in traditional Chinese medicine and an ingredient in health foods or dietary supplements for trace elements (Xie et al. 2006). Among 12 provenances at five sampling times, the most values of Fe, Mn and Cu concentrations were distributed in 0.2000–0.6000 (accounting for 70.1 % of all measured values), 0.0400–0.0700 (80.1%) and 0.0125–0.0175 (66.7%) mg·g^{-1}, respectively (Fig. 4). The most values of Zn concentration (accounting for 88.3%) were ranged from 0.0200 to 0.0400 mg·g^{-1}, showing a normal distribution. However, only 53.3% values of Se concentration were ranged from 0.0065 to 0.0170 mg·g^{-1}, while 43.3 % values of Se concentrations were below the 0.0065 mg·g^{-1}. The lower concentration of Se is likely to be associated with leaf development phases during the sampling time. Our results confirmed that microelement concentrations in leaves of *C. Paliurus* are very different from the various harvesting seasons (Xie et al. 2006). The Mn, Zn and Cu concentrations in *C. Paliurus* leaves in the present study are obviously lower than the results from Xie et al. (2006), where the range of Mn, Zn and Cu concentrations were 0.7120–0.7180, 0.0668–0.0685 and 0.0242–0.0406 mg·g^{-1}, respectively. However, the concentrations of Fe and Se are significantly higher than the results reported by Xie et al. (2006). These differences in microelement concentrations of *C. Paliurus* leaves are likely to be associated with genetic, cultural, harvesting, soil and climatic factors that occur during the growing period in each specific research. Compared to the results from alga and other plants, the concentrations of microelements in the leaves of *C. Paliurus* were notably lower than these in brown alga (*Eisenia arborea*) reported by Hernández-Carmona et al. (2009), but greater than these in medical herbs (Lovkova et al. 2001; Kalny et al. 2007), papaya (Hardisson et al. 2001) and rice grains (Jiang et al. 2008; Wang et al. 2009), and dill (*Anethum graveolens*), (Słupski et al. 2005).

Table 3. Pearson's correlation coefficients between the contents of measured microelements in the leaves of *Cyclocarea paliurus* (n=60)

Microelements	Item	Fe	Mn	Cu	Zn
Mn	Coefficient	-0.044			
	Significant	0.737			
Cu	Coefficient	-0.551**	-0.058		
	Significant	0.000	0.661		
Zn	Coefficient	-0.084	-0.027	0.594**	
	Significant	0.522	0.835	0.000	
Se	Coefficient	-0.642**	0.241	0.805**	0.368**
	Significant	0.000	0.063	0.000	0.004

Notes: ** Correlation is significant at the 0.01 level (2-tailed).

It is believed that the therapeutic effects of *C. Paliurus* are related to the physiologically active substances (PAS, such as phenolic compounds, polysaccharide, etc.), (Jiang et al. 2006; Zhang et al. 2010; Xie et al. 2010) and the enriched concentrations of several trace elements in the leaves (Xie et al. 2005). Metals also play an important role in cofactors or activators of enzymes for medicinal plants to synthesize and accumulate various types of PAS (alkaloids, phenolic compounds, terpenoids, etc.), (Lovkova et al. 2001). For example, Cu is involved in nitrogen metabolism and activates the synthesis of nitrogen-containing compounds, such as aromatic amino acids which serve as primary precursors of alkaloid PAS and phenolic compounds. Among measured 12 provenances, there were obvious differences in microelement concentrations in present experiment. Ranking of the total concentration of measured microelements (the sum of the concentration of the individual microelement) in 12 provenances was 0.9988 mg·g^{-1} (HJH)> 0.7332 mg·g^{-1}(JXS) > 0.5984 mg·g^{-1} (JLS)> 0.5847 mg·g^{-1}(GLP) > 0.5609 mg·g^{-1}(AQLF) > 0.5385 mg·g^{-1}(FZP) > 0.5319 mg·g^{-1} (GZY) > 0.5290 mg·g^{-1} (GJH)>0.5281 mg·g^{-1}(ASC) > 0.5305 mg·g^{-1}(ZAJ) > 0.5096

mg·g^{-1}(HHF) > 0.4451 mg·g^{-1}(YKM). Fe concentration in 12 provenances had almost the same ranking trend, which implied that the genetic variations provided opportunities to select provenances with rich contents of microelements.

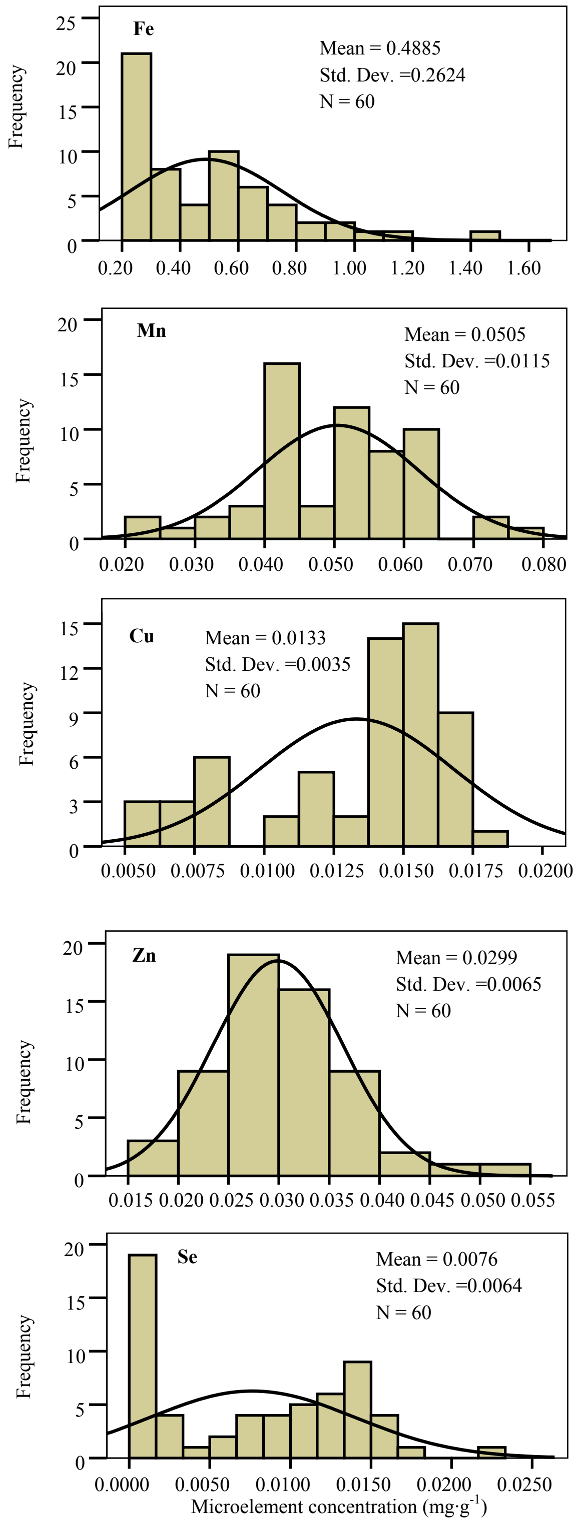

Fig. 4 Distribution of mean microelement (Fe, Mn, Cu, Zn and Se) concentrations in leaves of 12 *Cyclocarea paliurus* provenances at five sampling times (n=60).

In conclusion, our results clearly demonstrated that higher concentrations of microelements (Fe, Mn, Cu, Zn and Se) exhibited in leaves of *C. paliurus*, while the concentrations of microelements had a significantly difference among 12 provenances and at different sampling times. These results provided not only a sound basis for selecting provenances with high microelements, but also theoretical principles for plantation cultivation of *C. paliurus* in future. However, many factors (genetic, cultural, harvesting, soil and climatic factors) affect the microelement accumulation in the leaves, thus more studies are required to determine optimum cultivation patterns of *C. paliurus* plantations for producing health-promoting elements in food industry.

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